REGENERATION OF COENOSARC FRAGMENTS REMOVED FROM THE STEM OF TUBULARIA CROCEA

A. GOLDIN AND L. G. BARTH

(From the Department of Zoölogy, Columbia University, the Marine Biological Laboratory, Woods Hole, Mass., and Queens College, New York)

The rôle of environmental factors in the regeneration of hydroids has been studied extensively, the evidence all pointing to the extreme lability of hydroid systems. The literature on this subject has recently been reviewed by Barth (1940*b*). Morgan (1903) found that, when the cut end of a *Tubularia* stem was placed in sand, regeneration was inhibited at that end. Regeneration is inhibited also when the sectioned ends of a *Tubularia* stem are ligated. The reason for this inhibition was made clear by experiments in which *Tubularia* stems were exposed to a differential of oxygen in the sea water. Barth (1938*a*) was able to reverse the normal polarity by placing the distal end of the stem in a glass capillary, and he attributed this reversal to a lack of oxygen at the covered end. Miller (1937) reversed the polarity of *Tubularia* stems by exposure of the proximal end to a higher oxygen tension. Further, the rate of regeneration was shown to be dependent upon the oxygen tension (Barth, 1938*a*). All of these experiments have been interpreted as meaning that regeneration in *Tubularia* is dependent upon the availability of oxygen (Barth, 1940*b*).

Experiments designed to determine the origin of polarity in regenerating *Tubularia* stems are complicated by the presence in these stems of an already existing polarity. This polarity is evidenced by a gradient in the rate of regeneration and a gradient of oxygen consumption in the stems (Barth 1938*b*, 1940*a*), and by the dominance of distal over proximal levels (Barth, 1938*b*). Direct exposure of the coenosarc to sea water provides a sufficient stimulus for regeneration (Zwilling, 1939), and since the process of regeneration involves reorganization of cells, it was indicated that exposure of the entire coenosarc surface to sea water might result in sufficient reorganization to obliterate the existing gradients in the stem. The coenosarc fragments could then be subjected to carefully controlled environmental differentials in an attempt to determine the rôle of the environment in regeneration.

The experiments were therefore designed to ascertain: (1) the nature of the structural changes which occur during the development of coeno-

sarc fragments; (2) whether there is a gradient of oxygen consumption in coenosarc taken from different levels of the stem; (3) the polarity exhibited by coenosarc fragments during regeneration.

Methods

During June and July the experiments were performed on *Tubularia crocea* collected from the wharf piles at the Marine Biological Laboratory. From September through December colonies were collected from floats in the Far Rockaway channel in New York City. Uniform, clean stems 5 to 8 cm. in length were chosen for the experiments. Segments 10 mm. long were used, the hydranth plus the first 5 mm. being discarded. The cuts were made with iridectomy scissors. Holding the perisarc at one end of the stem segment with a jeweler's forceps, a needle was passed gently over the perisarc, and the coenosarc expressed at the opposite end. During the summer most satisfactory survival and regeneration were obtained when the coenosarc fragments were kept in running sea water which had been filtered through absorbent cotton. The coenosarc fragments were placed on agar (2 per cent agar in sea water) in Syracuse dishes, and the latter transferred to a large glass aquarium through which the filtered sea water constantly flowed. The fragments were kept one-half inch from the surface of the water by elevating the Syracuse dishes in the aquarium. This was done to insure the availability of oxygen. The coenosarc fragments were moved around in the dish every few hours to insure uniform healing. Twenty-four hours after removal, they were transferred to Syracuse dishes which contained no agar. The coenosarc fragments removed from stems collected at Far Rockaway during the fall and winter were very hardy, satisfactory viability and regeneration being obtained using filtered sea water. Agar and continuous circulation of the sea water were not necessary. The operations and observations

---

Explanation of Plate I [1]

Figs. 1–3, and 8–11 (× 15); Figs. 4 and 6 (× 160); Figs. 5 and 7 (× 950).

1. Coenosarc fragment (above) and empty perisarc (below) immediately after expression of the coenosarc.

2 and 3. Coenosarc fragment two hours (Fig. 2) and twenty-four hours (Fig. 3) after removal from the perisarc.

4 and 5. Section of coenosarc fragment immediately after removal.

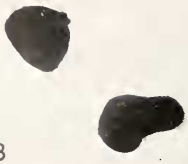
6 and 7. Section of coenosarc fragment two hours after removal.

8–11. The types of regenerants obtained from expressed coenosarc fragments. Unipolar (Fig. 8); bipolar (Fig. 9); bipolar-unipolar (Fig. 10); multipolar (Fig. 11).

[1] The authors wish to thank Mr. Jack Godrich for his assistance in the preparation of the photomicrographs.


1

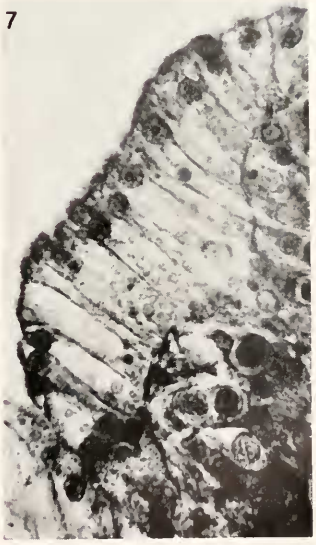

2


3


4


5


6


7


8


9


10


11

PLATE I

were made with the aid of a binocular microscope. The coenosarc fragments were fixed in Bouin's picro-formol-acetic fixative. They were sectioned at five microns, and stained with Delafield's haematoxylin.

The Development of Coenosarc Fragments

When coenosarc is removed from the perisarc, the tissues undergo a series of structural changes resulting, finally, in regeneration of hydranths. For convenience of description, the process may be divided into six stages based on characteristic morphological relationships.

Stage 1. The coenosarc has just been removed from the perisarc. There has been some morphological disturbance due to the mechanics of the operation. Plate I, Fig. 1, shows the condition of the coenosarc, above, and the empty perisarc, below. When examined histologically (Pl. I, 4), it may be noted that the ectodermal and endodermal layers are well defined, although the coelenteron has been obliterated. The nuclei of the ectodermal cells are centrally located and there is no trace of perisarc present (Pl. I, 5).

Stage 2. Two hours after removal the fragment has begun to contract along the original distal-proximal axis (Pl. I, 2), and the interior has become somewhat vacuolated (Pl. I, 6). The ectoderm is still well defined, but the nuclei of the ectodermal cells are located peripherally and the cells are somewhat elongated and swollen (Pl. I, 7). No perisarc is present. The atypical appearance of the ectodermal cells may be an indication of cellular degeneration, which is followed by a sloughing off of the original ectodermal cells. This loss of cells from the coenosarc may be observed, with the aid of a binocular microscope, from the time the perisarc is removed until a new perisarc is formed.

Stage 3. Twenty-four hours after removal, the coenosarc has undergone further contraction and is now somewhat spherical (Pl. I, 3). The outer layer is not well defined, and the inside of the spherical mass consists of numerous, closely packed cells (Pl. II, 12, 13). Traces of new perisarc may be noted around the periphery of the tissue mass (Pl. II, 13).

Stage 4. Thirty-six hours after removal, the center of the tissue

---

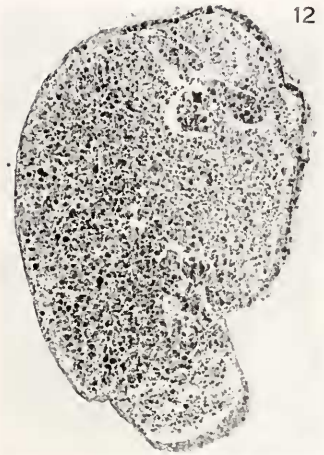
Explanation of Plate II

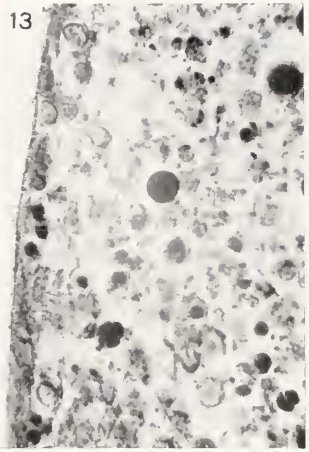
Figs. 12, 14, and 16 (× 160); Figs. 13, 15, and 17 (× 950).

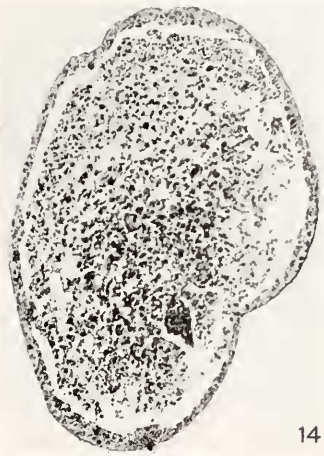
12 and 13. Section of coenosarc twenty-four hours after removal from the perisarc.

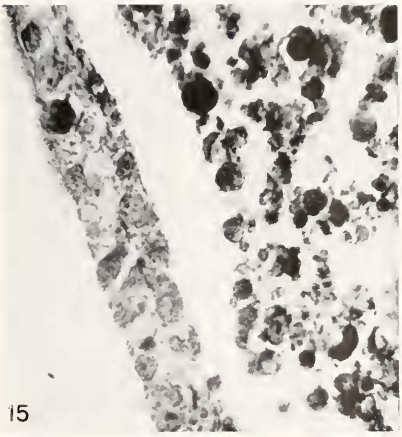
14 and 15. Section of coenosarc thirty-six hours after expression.

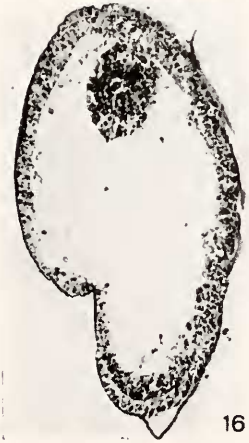
16 and 17. Section of coenosarc sixty hours after expression.











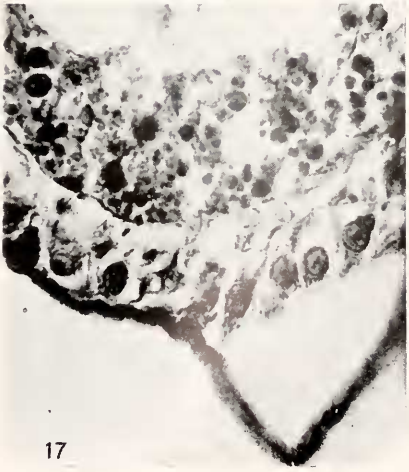


PLATE II

mass is less solidly packed with cells, and ectodermal cells have started to become arranged around the periphery (Pl. II, 14, 15).

Stage 5. Sixty hours after removal, the center of the mass of tissue is hollow. Well-defined ectodermal and endodermal layers have been formed around the periphery (Pl. II, 16, 17). The ectodermal cells are smaller than the ones in Stage 2 (Pl. I, 7).

Stage 6. Seventy-two to ninety-six hours after removal of the coenosarc fragments from the perisarc, regeneration occurs. This stage is characterized by the formation and emergence of new hydranths (Pl. I, 8–11). Hydranth formation is preceded by an aggregation of cells and later of pigment at the point of regeneration. Some of the fragments, although healthy in appearance, do not regenerate. They develop as far as Stage 5 (Pl. II, 16), at which time a heavy perisarc is secreted and development stops.

The changes which occur after removal of the coenosarc from the perisarc result in a general morphological dedifferentiation, namely, the formation of a spherical mass of coenosarc tissue in which the original ectoderm and endoderm are no longer clearly defined. Cellular dedifferentiation was not observed, no evidence being found that the cells return to an embryonic condition. The morphological dedifferentiation is followed by a redifferentiation, involving the reorganization of ectoderm and endoderm, formation of new perisarc, and subsequent regeneration. It is of interest to note that the time required for regeneration of the fragments is longer (72–96 hours) than the time required for intact stem segments (approximately 36 hours) at the same temperature. The additional time required for regeneration of the fragments is understandable when the time required for the initial dedifferentiation and early reorganization is taken into account.

The Rate of Oxygen Consumption of Coenosarc Removed from Different Levels of the Stem

In these experiments young unbranched stems were selected. The segments of stem used were 10 mm. long. The distal segments were taken from the region extending from 5 to 15 mm. proximal to the hydranth, and the proximal segments 20 to 30 mm. proximal to the hydranth. The coenosarc fragments were removed from these stem segments and kept in running filtered sea water until ready to be placed in the Warburg manometers. The fragments were placed in the manometers from 17 to 24 hours after removal from the perisarc. At this time the fragments have reached their greatest morphological dedifferentiation (Stage 3, Pl. II, 12). They have become spherical and are

protected from the mechanical shaking of the Warburg manometers by a thin, newly secreted perisarc. The rate of oxygen consumption was calculated on the basis of $mm.^3O_2$ used per hour per ten mg. of dry weight. The results are summarized in Table I. In most of the ex-

TABLE I

Oxygen consumption of distal and proximal coenosarc fragments removed from the perisarc. Rate = $mm.^3$ O_2/hr./10 mg. dry weight. The temperature of the sea water during the experiments was 18.5±.02°C.

Description of coenosarc fragments			Oxygen consumption			
Exp.	No.	Region	O_2	Time	Dry weight	Rate
			$mm.^3$	hours	mg.	
RE 1	19	distal	48.5	10.75	0.494	91.0
	19	proximal	37.2	10.75	0.391	88.5
RE 2	15	distal	19.8	9	0.46	48.0
	15	proximal	23.4	9	0.69	37.6
RE 3	4	distal	6.6	22	0.224	13.4
	14	proximal	41.2	22	1.294	14.4
RE 4	18	distal	10.5	9	0.720	16.2
	17	proximal	10.8	9	0.735	16.3
RE 5	14	distal	13.1	8	0.378	43.5
	15	proximal	15.2	8	0.482	39.5
RE 6	19	distal	24.3	7	0.729	47.7
	19	proximal	19.6	7	0.694	40.5
RE 7	15	distal	18.2	11.5	0.764	20.7
	15	proximal	17.0	11.5	0.721	20.4

periments the distal rates are approximately the same as the proximal rates. The averages of the distal and proximal rates for the seven experiments are 40.1 and 36.7 respectively. The distal and proximal regions of stem segments with perisarc, however, show a distal-proximal gradient in rate of oxygen consumption (Barth, 1940*a*). This difference in rate is present after the stems are cut, and persists from 24 through 36 hours after cutting. The distal and proximal coenosarc fragments, however, show only slight difference in rate 24 hours after removal from the perisarc. This means that the coenosarc fragments must have lost the differential during the first 24 hours. Thus, the reorganizational changes in the coenosarc fragments involve a dedifferentiation of the physiological gradient present in the intact stem.

Apparently then, exposure of the coenosarc to sea water has an effect on the rate of regeneration. In order to clarify the nature of this effect, the rates of distal coenosarc fragments and distal stem segments may be compared. The average rate of oxygen consumption of distal coenosarc fragments is 40.1 as compared with 21.8 for distal stem segments, the latter average being calculated from data presented by Barth (1940*a*). If the dry weight of the stem perisarc represents even as high as 50 per cent of the dry weight of the stem, the rate of distal coenosarc fragments would still be as high as the distal rate of the stems. Thus, the distal coenosarc fragments consume oxygen at a rate at least as high as the distal stems. Since, in addition, distal and proximal coenosarc fragments respire at about the same rate, the effect of exposure of the coenosarc to sea water and the resultant dedifferentiation is to increase the rate of oxygen consumption of coenosarc from proximal levels of the stem up to the higher rate of the distal levels.

Polarity of the Regenerated Coenosarc Fragments

The polarity relationships exhibited by the regenerated coenosarc fragments are worthy of examination for comparison with stems, where only unipolar and bipolar forms regenerate after cutting. Uniform exposure of the coenosarc to sea water removes the possible complication of an environmental differential created by the presence of the perisarc. The regenerated fragments may be classified using the general terminology employed by Child (1927) for *Corymorpha,* the groupings being based upon the axial pattern developed by the fragments. The regenerated coenosarc fragments may be classified as unipolar, bipolar, bipolar-unipolar, multipolar, and apolar. The unipolar forms have regenerated a single hydranth on the rounded mass of tissue (Pl. I, 8). Bipolar regenerated fragments have formed hydranths at two opposite poles of the coenosarc mass (Pl. I, 9). The bipolar-unipolar forms have regenerated two hydranths from the same region of the coenosarc (Pl. I, 10). These may be two independent hydranths or two partially fused hydranths. The category "multipolar" is used to designate fragments which have regenerated more than two hydranths. This group includes regenerated fragments ranging from tripolar forms, to forms in which the entire surface of the coenosarc has become covered with tentacles (Pl. I, 11). In each of these categories are included forms in which the regenerated hydranths are not complete. Thus, a hydranth may be lacking in the number of oral or basal tentacles or lacking in any of the structures necessary for a complete hydranth. For the purpose of this analysis, however, it was not deemed necessary to subdivide the various

categories with respect to these irregularities. Many of the hydranths which form do not emerge from the new perisarc. These may be readily classified in the above groups, so that no distinction is made between emerged and non-emerged hydranths. The apolar forms are those fragments which fail to regenerate. They develop as far as Stage 5 (Pl. II, 16), form a thick perisarc, and remain in that condition.

The classification of the regenerated coenosarc fragments is arranged in Table II. The unipolar and multipolar forms make up the greatest percentage of the regenerants (78.6 per cent). The bipolar and bipolar-unipolar forms comprise a much smaller percentage of the total (21.4

TABLE II

Classification of the regenerated coenosarc fragments obtained after the removal of the perisarc. The observations were made at 96 hours. Percentage regeneration equals the number of a particular kind of regenerated coenosarc fragment divided by the total number of fragments which formed hydranths. The temperature of the sea water was 19±2°C.

	Description of the regenerated coenosarc fragments				No regeneration	
	Unipolar	Bipolar	Bipolar-unipolar	Multi-polar	Apolar	Dead
Number	73	26	9	56	50	166
Percentage Regeneration	44.5	15.9	5.5	34.1		

per cent). Since exposure of the naked coenosarc to sea water is a sufficient stimulus for hydranth formation (Zwilling, 1939), the formation of hydranths should be enhanced when the entire coenosarc is naked, and therefore in more direct contact with sea water and oxygen dissolved in the sea water. That this may be true is demonstrated by the relatively high percentage of multipolar regenerants (34.1 per cent) obtained after removal of perisarc. These multipolar forms are never obtained when the perisarc is left intact. The appearance of a high percentage of unipolars (44.5 per cent) suggests that in these cases the mass of tissue may have been exposed to a uniform gradient of oxygen in the sea water, for many of them became attached to the bottom of the dish at an early stage and the hydranths always formed away from the attached end. This interpretation is supported by Child's experiments with *Corymorpha* (1928) in which hydranths were regenerated at the upper surface of undisturbed cell aggregates. Coenosarc fragments develop in the same way, irrespective of the level of the stem from which they are removed.

Data for the regeneration of coenosarc fragments removed from three different levels of the stem are summarized in Table III. The coenosarc fragments of all three levels of the stem give rise to regenerants having similar types of polarity relationships. In addition, it may be noted that 96 hours after removal of the coenosarc from the perisarc, approximately

Table III

Classification of the regenerated coenosarc fragments removed from different levels of the stem. The observations were made at 96 hours after removal of the fragments from the perisarc.

	Description of the regenerated coenosarc fragments					No regeneration	
	Unipolar	Bipolar	Bipolar-unipolar	Multi-polar	Total	Apolar	Dead
Distal	10	4	2	12	28	14	18
Middle	12	3	1	13	29	19	12
Proximal	15	6	2	12	35	19	6

the same number of regenerants appear at all three levels. Thus, the gradient in the rate of regeneration present in *Tubularia* stems (Barth, 1938*b*, 1940*a*) apparently disappears when the coenosarc fragments are removed from the perisarc.

Discussion

One of the chief difficulties in any attempt to analyze the rôle of the environment on regeneration is the inability to work with homogeneous systems. That hydroids have a gradient of metabolic activity has been well established. This gradient is developed, apparently as the result of an environmental differential, early in the development of the organism. Thus, Child (1925) has shown that a metabolic gradient is probably established as a result of the nature of the orientation of the egg during its growth. Further, once a gradient has been established, it may maintain itself in a uniform environment, and the gradient has therefore become a function of localized differences within the tissues themselves. Barth (1938*b*) demonstrated a gradient in the rate of regeneration along the length of the stem of *Tubularia*, distal segments regenerating at a higher rate than proximal segments. There is likewise a gradient of oxygen consumption of the parts of the stem (Barth, 1940*a*). Barth (1938*b*) also suggested that the dominance of distal over proximal levels of *Tubularia* stems might be interpreted as a compe-

tition for substance "S," the success of which is dependent upon the activity of enzyme "E." Thus, an organism living in a uniform external environment may nevertheless maintain its own heterogeneity once this heterogeneity has become established.

In order to determine more accurately the rôle of environmental factors on the origin of new gradients in regeneration, it is of importance to obliterate first any existing gradients in the animal tissues themselves. Some attempts in this direction have been made. Child (1928) found that cells of *Corymorpha*, when dissociated mechanically, will aggregate and establish new polarity relationships. *Corymorpha* stems, when subjected to toxic agents, may lose their established polarity relationships and form new gradients of regeneration (Child, 1927), the new gradients being produced by a differential exposure to the environment.

The experiments with expressed *Tubularia* coenosarc indicate an obliteration of the original polarity after the coenosarc is removed from the perisarc. This is borne out by the reorganizational changes which the coenosarc undergoes after removal. There is, at first, a morphological dedifferentiation, in which the mass becomes spherical and the ectoderm and endoderm are no longer clearly defined. Cellular dedifferentiation, however, was not observed. The morphological dedifferentiation is followed by a redifferentiation. The ectoderm and endoderm are reorganized and a new perisarc is formed. Subsequently, regeneration occurs. If the polarity of an organism is dependent upon a gradient of activity of some enzyme, as suggested by Barth (1938*b*), then it is quite likely that the activity of this enzyme is radically changed during the process of reorganization. That the initial polarity is lost, is evidenced also by the appearance of regenerated hydranths at the free surface of fragments which have become attached to the bottom of the dish, the regenerated hydranths having no necessary relation to the original polarity. It is further substantiated by the appearance of a high percentage of multipolar forms in which appear numerous and apparently unrelated polarity relationships. The appearance of these multipolar forms must mean that the original polarity is no longer extant; the exposure of the coenosarc to the sea water being sufficient to stimulate regeneration at many points on the uniform mass of tissue.

Correlated with the disappearance of the original polarity is the disappearance of the difference in oxygen consumption of tissues removed from distal and proximal levels of the stem. The sharp gradient of oxygen consumption found along the length of the stem (Barth, 1940*a*) is not exhibited by the excised fragments at the time when morphological dedifferentiation has reached its peak (17–24 hours after removal of the tissue). At this time the rates of oxygen consumption of dedifferenti-

ated coenosarc fragments from both distal and proximal levels of the stem are at least as high as the rate for distal stem segments. The loss of the gradient of oxygen consumption is due, therefore, to a general increase in rate. This increase in rate is probably stimulated by a high availability of oxygen to the coenosarc fragments, made possible by their removal from the perisarc. Thus, the removal of the coenosarc from the perisarc results in a reorganization involving not only morphological dedifferentiation but also a dedifferentiation of the physiological gradient. The end product of this process is a more homogeneous mass of *Tubularia* cells. The localized differences in the ability to regenerate, found in stems covered with perisarc, also disappear as a result of the morphological and physiological dedifferentiation. Coenosarc fragments removed from distal and proximal levels of the stem regenerate at the same rate and develop similar types of polarity relationships.

Expressed coenosarc, therefore, if used at the time when dedifferentiation is greatest (approximately twenty-four hours after removal from the perisarc), offers good biological material for studies of the environmental factors stimulating regeneration and for an investigation of the origin of polarity in regeneration.

Summary

The morphogenesis of coenosarc expressed from the perisarc of *Tubularia* stems is described. A series of structural changes occurs in the coenosarc, there being first a dedifferentiation of histological structure, followed by a redifferentiation culminating in the regeneration of new hydranths.

The gradient of oxygen consumption present in the stem of *Tubularia* disappears when the coenosarc is removed from the perisarc. This physiological dedifferentiation represents an increase as well as an equalization of oxygen consumption by coenosarc fragments from distal and proximal levels of the stem.

Concomitant with the morphological and physiological dedifferentiation, differences in the ability of distal and proximal levels of the stem to regenerate disappear. Distal and proximal coenosarc fragments regenerate at the same rate and develop similar types of polarity relationships.

The different kinds of regenerants obtained are described and classified on the basis of their polarity relationships. Evidences were given that these polarity relationships are new, and have no relation to the original polarity in the intact stem.

The value of using expressed coenosarc to study the effect of the environment on regeneration and on the origin of polarity in regeneration is discussed.

LITERATURE CITED

BARTH, L. G., 1938*a*. Oxygen as a controlling factor in the regeneration of Tubularia. *Physiol. Zoöl.*, **11**: 179–186.

BARTH, L. G., 1938*b*. Quantitative studies of the factors governing the rate of regeneration in Tubularia. *Biol. Bull.*, **74**: 155–177.

BARTH, L. G., 1940*a*. The relation between oxygen consumption and rate of regeneration. *Biol. Bull.*, **78**: 366–374.

BARTH, L. G., 1940*b*. The process of regeneration in hydroids. *Biol. Rev.*, **15**: 405–420.

CHILD, C. M., 1925. The axial gradients in Hydrozoa. VI. Embryonic development of hydroids. *Biol. Bull.*, **48**: 19–36.

CHILD, C. M., 1927. Modification of polarity and symmetry in Corymorpha palma by means of inhibiting conditions and differential exposure. *Jour. Exper. Zoöl.*, **47**: 343–383.

CHILD, C. M., 1928. Axial development in aggregates of dissociated cells from Corymorpha palma. *Physiol. Zoöl.*, **1**: 419–461.

MILLER, J. A., 1937. Some effects of oxygen on polarity in Tubularia crocea (abstract). *Biol. Bull.*, **73**: 369.

MORGAN, T. H., 1903. Some factors in the regeneration of Tubularia. *Arch. f. Entw.-mech.*, **16**: 125–154.

ZWILLING, E., 1939. The effect of the removal of perisarc on regeneration in Tubularia crocea. *Biol. Bull.*, **76**: 90–103.